GREEN SYNTHESIS OF ZINC NANOPARTICLE BY MICROBES

A THESIS SUBMITTED FOR THE PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE
IN
LIFE SCIENCE
BY

UPASANA PATTANAYAK
Roll No.:411LS2060

Under the Supervision of
DR. SUMAN JHA

DEPARTMENT OF LIFE SCIENCE

NATIONAL INSTITUTE OF TECHNOLOGY

ROURKELA-769008, Orissa, India.

NATIONAL INSTITUTE OF TECHNOLOGY, ROURKELA, ODISHA, INDIA.

Certificate

This is to certify that the thesis entitled "**GREEN SYNTHESIS OF ZINC NANOPARTICLE BY MICROBES**", submitted to National Institute of Technology, Rourkela for the partial fulfillment of Master Degree in Life Science during session 2011-2013 in Department of Life Science is an authentic work and a record of bonafide research work carried out by **Upasana Pattanayak (411ls2060)** under my supervision and guidance. To the best of my knowledge, the thesis fulfills part of the requirements for the award of Master of Science in Life Science. The matter and result embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

Dr. Suman Jha

Department of Life Science

National Institute of Technology, Rourkela.

DECLARATION

To the best of my knowledge the research project report entitled "**GREEN SYNTHESIS OF ZINC NANOPARTICLE BY MICROBES**" reported here in its original and has been submitted to National Institute of Technology, Rourkela for partial fulfillment of the degree of Master of Science in Life Science is a bonafide record of the project work carried out by me under the supervision of Prof.Dr. Suman Jha,Assistant Professor, Department of Life Science, National Institute of Technology, Rourkela. This thesis is my own work and that, to the best of my knowledge and belief, the matter and results of this thesis has not been submitted by any other research persons or any students.

I do hope,this project work will satisfy our beloved teachers. I solicit kind and favourable appreciation from my teachers.

Dedicated to my parents and my beloved one…

ACKNOWLEDGEMENTS

I would like to take this opportunity to express my gratitude towards people who have helped me fare through the expedition of my research project. At the outset, I wish to express my deepest gratitude and sincere thanks to Prof. Dr. Suman Jha for providing me a chance for research project in his laboratory and providing me an opportunity to work under his guidance. His kind attention, care & continuous encouragement have been a source of inspiration for me throughout the project. I would like to acknowledge the excellent guidance, encouragement and support of my thesis advisors Prof.Dr.Suman Jha throughout my thesis endeavor. It is only with their mentoring, leadership and cooperation that I was able to finish this thesis. I am also grateful and offer my sincere thanks and gratitude for his constant guidance and indebted to him for sparing his valuable time for teaching me all about the Molecular Biophysics and all the biophysical techniques. His untiring effort also deserves special mention here without whom the fulfillment of this entire project would have been impossible.

I also pay my thanks to my co-guide Mr.Manoranjan Arakha, whose constant support and patience in guiding me throughout the project work and helped me to complete the project. I am highly indebted to him for sparing his valuable time for teaching me all the structural techniques. I am obliged and thankful to him for providing me the opportunity to gain knowledge and understanding of working skills of the aspects of my work from him.

I find this an appropriate chance to thank Prof. Dr. Samir Kumar Patra, HOD of Department of Life Science for providing me continuous encouragement and support which has been a source of inspiration for me throughout the project. I am also grateful and offer my sincere thanks and gratitude to him. I am also grateful Prof.Dr.Sujit Kumar Bhutia for providing me encouragement and support for the completion of my work. I would like to acknowledge the encouragement, support and help of Prof. Dr. Bibekanand Mallick and Prof. Dr. Surajit Das. I would also like to express my whole hearted gratitude to Prof. Dr. RasuJayabalan and Prof. Dr. BismitaNayak. I am also grateful to entire research team of National Institute of technologyfor their moral support.The constant support and encouragement that I got from them, has made my stay in NIT, Rourkela a memorable one.

I am highly indebted to Ms. Swayamsiddha Kar, PhD scholar for teaching me some basics of Molecular Biology, constant support, help and encouragement regarding my study. I also pay my thanks toMr. Pradipta Ranjan Rauta and Mr. Debasis Nayak, PhD scholar whose patiencein my study and their encouragement. I would like to acknowledge and thankful to Mr. Hirak Ranjan Dash, PhD scholar for his constant help and support.

I consider it a privilege to express my gratefulness to Prof .Dr. Debasis Chaira, Department of Metallurgical & Materials Engineering for giving me chances for the SEM analysis for my research project work in SEM laboratory in his department.I am highly indebted to Mr. Arindam Pal, Laboratory Technician of XRD laboratory, Department of Metallurgical & Materials Engineeringfor giving me chances for the XRD analysis. I also pay my thanks to Prof. Dr. Raj Kishore Patel, Department of Chemistry for giving me chances for the FTIR analysis. I am also grateful and offer my sincere thanks and gratitude to Mr. Jyoti Prakash Dhal, PhD scholar, Department of Chemistry for sparing his valuable time for teaching and helping me for the FTIR analysis.

Lastly, I would like to thank and dedicate my father Mr. Rashmi Ranjan Pattanayak, my mother Mrs. Archana Pattanayak, my sister and my beloved one whose constant source of inspiration stood behind me to give every kind of support and help, without whom this project would have ever been successful. I am also highly thankful to my uncle Mr. Amiya Das Pattanayak whose source of inspiration and blessings stood behind me to give every kind of support. This would not have been a successful effort without their blissful inspiration and unfailing emotional support. And finally, I would like to express my gratitude to my grandparents (Late. Sudhir Das Pattanayak and Late. Kamala Mohanty) whose source of inspiration, blessings and guiding light stood behind me to give every kind of support and success for my life. Above all, I am thankful to The Almighty God whose benediction has been my guiding light through out the days and always.

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UV-Visible	UV-Visible spectrophotometer
SEM	Scanning Electron Microscope
XRD	X-Ray diffraction
FTIR	fourier Transform Infrared Spectroscopy
NMR	Nuclear Magnetic resonance
SAW	Surface Acoustic Wave
LEDL	Light Emitting Diode
Nm	Nanometer
cm^{-1}	$centimeter^{-1}$
Fe	Ferrum (Iron)
Ni	Nickel
Co	Cobalt
Mn	Manganese
Zn	Zinc
SiO2	Silicon dioxide
TiO_2	Titanium dioxide
ZnO	Zinc oxide
MgO	Magnesium oxide
CaF_2	calcium fluoride
BN	Boron nitride
GaN	Gallium nitride
DMF	N, N-dimethylformamide
PVP	Poly N-vinyl pyrrolidine
TFATEB	tetra-n-tetra-fluroborate
CTAB	Cetyltrimethyl ammonium bromide
PEG-HCC	Polyethylene glycol-hydrophilic carbon clusters
FDA	Food & Drug Administration

MBC	Minimum bactericidal concentration
MIC	Minimum inhibitory concentration
ROS	Reactive Oxygen Species

ABSTRACT

Nanoparticles can be synthesized by using microbes and by plant extracts. Under the favorable conditions, size and surface of nanoparticles can be easily altered to achieve both passive and active transportation of drug. It can be projected forthe specific sites by attaching ligands to nanoparticle surface. Nanotechnology is recognized as the study of particle with a minimum of one dimension in nanometers. Synthesis of nanoparticles can be done through three methods like physical, chemical, and green synthesis methods. Green synthesis can be done using microorganisms, enzymes, or plant extracts. This project work is based and focused on the green synthesis method due to its non-toxic and ecofriendly process. In this project, Zinc (II) ion solution is reduced into ZnO nanoparticles using microbes like *Bacillus thuringenesis* and *Bacillus species*. Characterization of Zinc nanoparticles was done by UV-Visible spectrophotometer for its Surface Plasmon resonance, SEM for morphologies, XRD and FTIR for its crystalline nature.

Key words: Nanoparticles, Nanotechnology, nanometer, ligands, green synthesis, enzymes, plant extracts,UV-Visible spectrophotometer, surface Plasmon resonance, crystalline, SEM, XRD and FTIR.

CHAPTER 1

INTRODUCTION

1. INTRODUCTION

New technologies often create new challenges to science in addition to their benefits, raise concerns about health and various environmental problems.Recent nanotechnology holds a promise and a broad aspect towards wide applications of nanoparticles in a multiple way ofemerging fields of science and technology. Over last decades, nanotechnology has established as the great innovation of science and technology. Nanotechnology is a science and engineering branch of recent well established technology refering at the nanoscale,i.e. 1 to 100 nm. Generally, metal oxide nanoparticles are inorganic.Various nanoparticles like Fe, Ni, Co, Mn, Zn,etc. are known as the enormously accepted magnetic materials for awide range of applications like various electronic ignitionsystems, generators, vending machines, medical implants,wrist watches, inductor core, transformer circuits, magneticsensors and recording equipment, telecommunications,magnetic fluids, microwave absorbers, etc.They are also applicable in otherhigh-frequency applications [1, 2].The vast applications of nanoparticles in medical sciencesare drug delivery, imaging and diagnosis. Nanoparticles possess high surface to volume ratio due to its small size, which gives very distinctivefeatures to nanoparticles.A multiple of researchhave established that zinc oxide nanoparticles have antifungal and antibacterial activity. The other metal nanoparticles like silver nanoparticles also carrythese properties.

Recentinspiringimprovements in the field of nanotechnology have been observed because of development of different and efficientmethodologies to fabricate nanoparticles of particular shape and size depending on the specific requirements. At present, there is anemergent need to develop environmentally benevolent nanoparticles synthesis routes,which can be proceeded by biological method instead ofusing toxic chemicals. The use of ecologically beneficial materials like plant leafextract, bacterial cell extract, fungi and enzymes for thesynthesis of zinc nanoparticles proposes abundant benefits in terms of ecofriendlinessand compatibility for a wide range of pharmaceuticals.Accordingly, the researchers in the field of nanoparticles synthesis and assembly have turned to biological systems for inspiration [3].Currently, the use of “green” methods for synthesis and production of engineered nanomaterials in both industrial application and the scientific research has achieved a massive amount of interests [4]. Proper utilization of environmentally benevolent solvents and nontoxic chemicals are some of the key issues in green synthesis approach deliberations[5].The green synthesis method plays a significant role on the effective consumptions of nanoparticles.Predictable chemical methods for the production of nanoparticles are time consuming and have high manufacturing cost.

Surprisingly many organisms of both unicellular and multicellularnature are identified to produce reduced inorganic materials either intracellularlyor extracellularly. Some microorganismshelps in the synthesis of inorganic materials that includesmagnetotactic bacteria for the synthesis of magnetite nanoparticles, diatoms helps in the synthesis of the siliceous materials and S-layer bacteria helps in the production of gypsum and calcium carbonate layers.Multicellular organisms produce hard inorganic-organic composite materials like bones, shells and spicules using inorganic materials to build a complex structure.These biominerals are composite materials and consist of inorganic component capped with special organic matrix such as proteins, lipids, and polysaccharidesthat controlsmorphology of the inorganic compound. Advanced nanomaterials approaches led to the highly developed biomimetic assays [3].

Currently, countlessadvance biotechnological applications like remediation of toxic metals are being done usingmicroorganisms like bacteria and yeast.The detoxification occursfrequentlythroughthe reduction of metal ions or formation of the metal sulphides.Synthesis of various nanoparticles by microorganismsisused more than the other techniques, due to itsecofriendlynature to the nanoindustries. Different types of species of bacteria have tendency to synthesis metal nanoparticles intracellularly and their alloys respectively[3]. Uses of different species of plants for the synthesis of nanoparticles are also beneficial for our environment, which is through the biological processes by eliminating the sophisticated process of maintaining the bacterial cultures.

Nanosized materials have already been comprehensively studied by the researchers worldwide, because of their unique physical properties like electrical conductivity, optical band gap, refractive index, magnetic properties and superior mechanical properties for example hardness of nanomaterials, and chemical properties compared with their counterpart bigger structured materials [6].Nanosized materials are alsoknown as novel antimicrobial agents. The antimicrobial agents governby high surface area to volume ratio that isbeing the contemporaryattentionfor the researchers due to increasing and progressing microbial resistancesagainst various metal ions, various antibiotics, and the development of resistant strainsin multiple ways[7].

The antimicrobial property of nanoparticles is determined by their size, shape, morphology, composition and crystallinity. Researchers are going on to synthesize nanoparticles,designing of nanodevices and application of these in different fields like medical science, environment, energy, information, and communication, industries and also in food technology etc.

Generally there are two parts of nanoparticles, the core material and a surface converter. The surface converter is responsible for alteration in the physicochemical properties of core materials. The core material consists of a variety of biological materials like phospholipids, lipids, lactic acid, chitosan, and dextran or may be formed of carbon, chemical polymers, silica or metals. Various chemical compounds, drugs, probes and proteins are attached to the surface of nanoparticleswith the help of non-covalent interactions or by physical adsorption. When the chemical compounds attach to the surface of the nanoparticles, the physiochemical properties changes. Accordingly the functionality of nanoparticles is enhanced or changed. The efficiency of nanoparticles for various applications depends upon the physiochemical characteristics of the core material and the surface modifiers. The composition of core materials along with surface modifiers may makes the nanoparticles biodegradable and biocompatible. Toxicity of nanoparticles, nanotoxicity can be reduced by modification of their surface properties at the time of nanoparticles use for drug carriers.

Zinc oxide nanoparticles have been extensively used in many industrial areas such as solar cells, UV light-emitting devices, gas sensors, photo-catalysts, pharmaceutical and cosmetic industries [8-12].It is also known as a versatile semiconductor material which has been a center of attention andinterest for its broad range of applications in many areas like biosensors, biological labels, electrochemical cells, varistors, Ultraviolet (UV) photodiodes, electrical and optical devices, and surface acousticwave (SAW) devices. ZnOnanoparticle is inexpensive and economical. It is a n-type semiconductor of wurtzite structure.It also has the optical transparency in the visible range. The visible light emission of zinc oxideis of immensesignificance for white-light LEDs [13].This is a metal oxide nanoparticle. The metal nanoparticles have the surface plasmon resonance properties in UV-visible region, nontoxic, self-cleansing [14]. ZnO is nontoxic and it is a strong antibacterial agent.ZnO nanoparticles have bactericidal effects on both gram-positive and gram-negative bacteria [15]. They also have antibacterial activity against spores that are resistant to high temperature and high pressure. The antimicrobial agents are natural or synthetic compounds which are helpful in the inhibition of microbial growth. Various classes of antimicrobial agents are used in the textile industry, most of which are biocides [16]. The increased production and utilization of ZnO nanoparticles enhances the probability of exposure in occupational and environmental surroundings.Various types of heavy metals and the metal oxides either in their free state or in compounds at very low concentrations are toxic to microbes [17]. The studies about toxicity have shown that zinc ions do not cause any damage to the DNA of human cells. The antibacterial activity of ZnO nanoparticles is due in part to their electrostatic interaction with cell surfaces which shows that on contact with bacteria, the cytotoxic actions of ZnO nanoparticles ruptures the lipid bilayer of bacterium, resulting in leakage of the cytoplasmic substances [16].

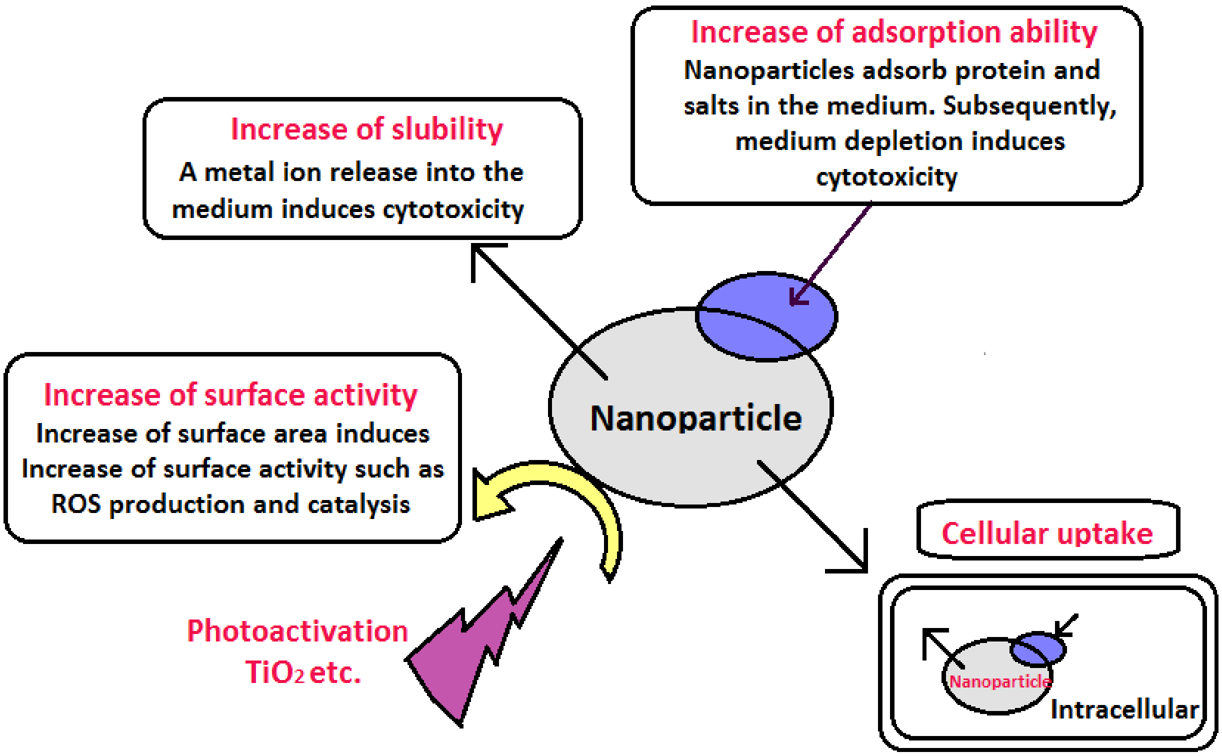

Fig. 1. Cytotoxicity of nanoparticles in case of the in vitro study

Otherwise, the bacteria are the primary and principal contributors to the biodiversity on earth and mediate environmental processes that sustain life.Thus, there is a duality to bionanotechnology research. Another principal aspect is to develop nanotechnology for the antibacterial applications which is the recent researchfocus.This may also be helpful for the study of nanomaterialsimpact on bacterial ecology.

From the above illustrated information the current study is focused upon the foremost objective of evolving a simple green method for ZnO nanoparticles fabrication, its physio-chemical characterization and antimicrobial activity.

CHAPTER 2

LITERATURE SURVEY

2. LITERATURE SURVEY

2.1. Nanoscience and Nanotechnology

Nanotechnology is defined as a highly developing field because of its vast array of applications in various fields of medical science, technology and various research areas. The word 'Nano'isoriginated from a Greek word whose meaning is extremely small or dwarfs [18]. The basic concepts behind nanoscience and nanotechnology was reported with a talk entitled "There's a Plenty of Room at the Bottom" by the physicist Richard Feynman at the California Institute of Technology (CalTech) on December 29, 1959 [19].The term "Nanotechnology" was later coined by Professor Norio Taniguchi by using Feynman's explorations of ultra precision machining.In 1974, the term nanotechnology was pioneered by Nori Taniguchi on the talk of Production Engineering at the Tokyo International Conference. He focused on the ultra precision machining in his talk and hisresearch was based on the mechanisms of machining hard and brittle materials like ceramics of silicon, alumina and quartz crystalsby ultrasonic machining.Richard Feynman established a talk on the scanning electron microscope that, it could be developed in resolution and stability whichwill bevaluable and functional foranyone to "see" the atoms.Scanning tunneling microscope helps in the proper detection and identification of individual atoms from whichthe modern nanotechnology began which was developed in 1981.Feynman also continued to identify the ability to arrange atoms which iswithin the bounds of chemical stability for the minute structures which in turn would lead to synthesis of materials in molecular and atomiclevels.

The study about Nanoscience and nanotechnology provides the well developedapplication of exceptionallyminiature things and be capable of the encroachment of all the fields of scientific research and development likePhysics, Chemistry, Materials andMetallurgy engineering, Biology and also in Biotechnology [18, 20].Bionanotechnology is the conjunction between biotechnology and nanotechnology for developingvarious biosynthetic and environmental ecofriendlytechnologies for the synthesis of various nanomaterials [21]. Multiple of research on synthesis of nanoparticles has put forth great interesttowards the emerging field of science due to their distinguishing physical and chemical properties than the macroscopic particles[22].The vast array of development of novel synthesis protocols and various characterization techniques are the evidences for the vastadvancement for the nanotechnology[23].

A brief and general definition of nanotechnology by the US National Science and Technology Council [24] states: “The essence of nanotechnology has the capability to work at the molecular level such as atom by atomfor the creation of large structures with essentiallyinnovative molecular organization. The aim is to exploit these properties by gaining control of structures and devices at atomic, molecular, and supramolecular levels and to become skilled atwell-organized manufacture and use these devices.” The United States National Science Foundation (USNSF)[24] defines nanoscience or nanotechnology is the study which deals with materials and systems deserving the following key properties:

1. The dimension must be at least one dimension from 1-100 nm.

2. The process can be designed with various methodologies whichshowelementarycontrol over the physical and chemical properties of structures that can be measure by the molecular-scale.

3. According to the building block property,larger structures form by the combination of smaller one. According to the Microbiological study, the Nanoscienceleds to the sizes ofdifferent types ofbioparticleswhich deals withbacteria, viruses,enzymes, etc. fall within the nanometer range.

Nanotechnology has the capability at atomic precision which is useful formaking of materials, various instruments and systems[24, 25]. Nanotechnology is the science and engineeringof recent well established technology which refers at the nanoscale.It is concerning 1 to 100 nanometersin size [26]. Nanotechnology can also be defined as the study and investigation about the synthesis, characterization, exploration and application of nanosizedmaterials which is of 1-100nm in size and will be valuable and functional for the development of science.A recent advance in the emerging field of nanotechnology has thecapability for the preparation of the highly ordered nanoparticulates of various different size and shape which led to the development of new biocidalagents [27]. The prefix nano means a factor of one billionth (10^{-9}) and can be applied, e.g., to time (nanosecond), volume (nanoliter), weight (nanogram) or length (nanometer or nm). In thiscommon use "*nano"*refers to the length and the nanoscale refers to a length from the atomic level of around 1nm upto 100nm.

Nanotechnology is useful in the techniques for various diagnostic processes, drug delivery, sunscreens, antimicrobial bandages, disinfectant and a friendly manufacturing process which reduces waste products thateventuallymost important to atomically precise molecular manufacturing with less waste products which is also serves as catalyst for greater efficiency in present manufacturing process by minimizing or eliminating the use of toxic materials for reduction of pollution such aswater and air, and, as an alternative energy production which issolarand fuel cells [21].The goal of nanotechnology is to close sizegap between the smallest lithographically fabricated structures and chemicallysynthesized large molecules.

The successful use of nanotechnology in the food industry can take a number of forms. These include the use of nanotechnology in packaging of materials. It is usefulfor developing the antimicrobial packaging of the food products. The nanoparticles are dispersed throughout the plastic and are capable to block reachingoxygen, carbon dioxide and moisture from fresh meats or other foods. Packaging can able to contribute the control of microbial growth in foodstuffswhich can lead to spoiling or in the case of a range of pathogenic microorganisms and appearance of disease due to spread of infection[28].They are also being investigated for their potential use as fungicides in agriculture, as anticancer drugs, and imaging in biomedical applications [29]. An additional advantage of nanobiotechnology is the development of consistent and more reliable processes for thesynthesis of nanomaterialsin excess of a range of sizes with superiormonodisperse character and chemicalcomposition.

2.2. Nanomaterials

The improvement of reliable experimental and investigational protocols for the synthesis of nanomaterialsmore over a range of chemical compositions, sizes and high monodispersity is one of the most challenging issues in current nanotechnology.The term 'Nanomaterial' is now frequently used for the conventional materials which are consciously and deliberately engineered to nanostructure of modern application of nanotechnology. Nanomaterials are the forms of matter at nanoscale. Generallynanomaterials are referred as the infrastructure or building blocks element for nanotechnology.The "Building blocks" for nanomaterials consist of carbon-based components and organics, semiconductors, metals and metal oxides.Nanomaterials with structural features at the nanoscale can be found in the form of clusters, thin films, multilayer and nanocrystalline materials which are often expressed by the dimensionality of 0, 1, 2 and 3. The materials include metals, amorphousand crystalline alloys, semiconductors, oxides, nitride and carbide ceramics in theform of clusters, thin films, multilayer and the bulk nanocrystalline materials, etc.

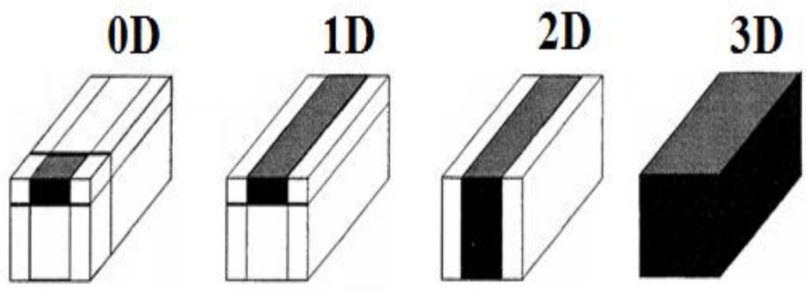

Fig.2. Classification of nanomaterials according to the dimension

Table.1.Classification based on dimensionality

Dimension	Example of nanomaterials
0D	Colloids, nanoparticles, nanodots, nanoclusters
1D	Nanowires, nanotubes, nanobelts, nanorods
2D	Quantum wells, super lattices, membranes
3D	Nanocomposites, filamentary composites, cellular materials, porous materials, hybrids, nanocrystal arrays, block polymers

The familiar nanomaterial ‘carbon black’ was used in industrial production over a century ago. The other early nanomaterials are fumed silica, a form of silicon dioxide (SiO2), titanium dioxide (TiO_2) and zinc oxide (ZnO). Nanomaterials can have different properties at nanoscale which is also established by the Quantum effects. Among all nanomaterials, some are having better conductivity towards heat and electricity, different magnetic properties, light reflection and also change colors according to their size which is also changed. These properties are a little bit different from the bulk materials. Nanomaterials also have larger surface areas than similar volumes of larger-scale materials which signify the meaning of more surfaces is available for interactions with other materials around them.Nanomaterials are being controlled to nano crystalline size which is less than 100 nm which can show atom-like behaviors. This results from its higher surface energy due to their large surface area and wider band gap between valence and conduction band. It occurs when they are divided to near atomic size [26, 30]. Nanomaterials are also referred as ‘‘a wonder of modern medicine’’. It signifies the importance of antibiotics which kill atleast six different disease-causing organisms whether the nanomaterials can kill atleast 650 cells [31].Nanomaterials are being enthusiastically researched for specific function like microbial growth inhibition,carriers of antibiotics and also act as killing agents [28].

2.3.Metal oxide nanoparticles

Different types of nanoparticles developed by the chemical and physical methods show poor morphology.Mostly toxic chemicals are used in these processes and elevated temperaturewhich isbeing helpfulfor the synthesis of respective nanoparticles are toxic to our environment[18,32].Metal oxides have also been serves as sorbents for various environmental pollutants. But the biological methods of synthesis are more favourable than the chemical and physical methods of synthesis since these methods are eco-friendly.The biological methods for synthesisof nanoparticles by using various microorganisms, enzymes, plants,and their extracts have been suggested as the probable and promisingecofriendly alternatives to the chemical and physical methods of synthesis [30]. The biocompatibility of nanoparticles is more essential for specific biomedical applications and researches [33].

The metal nanoparticles have various functions which are not observed in bulk phase [34, 35]. All these arealreadybeen studied broadlydueto their exclusive electronic, magnetic, optical, catalyticaland antimicrobial properties of [36, 37] wound healing and also for anti-inflammatory properties [38].The metal nanoparticles have the surface plasmon resonance absorption in the UV–visible region [30]. Over the past few decades, the structure of inorganic nanoparticles exhibit appreciable, drastic, novel and highly improved physical, chemical and biological properties with well recognized function due to their nanoscale size [27]. Recent studies reported that nanoparticles of some materials including metal oxides can also induce the cell death in eukaryotic cells [39-42]and the growth inhibition in prokaryotic cells due to cytotoxicity nature. The transition metal oxides with nanostructure and semiconductors with dimensions in the nanometer have capacity to attract towards the areas belonging to Physics, Chemistry, Materials and Metallurgy engineering, Biotechnology, Information technology and Environmental science [30] with their respective technologies in several aspect of development and research. Different types of specific applications can be usefor synthesis of metal oxide nanoparticles as major components like sensors, pigments and various medical materials.The dispersion of metal oxide nanoparticles in physiological solutions is also important for biological in vitro and in vivo studies [43].

2.3.1. Semiconductor nanoparticles

Semiconductor nanoparticles are the focus ofimmenseinterest due to their size-dependent physical propertiesand potential applications in miscellaneous areas such asbiomedicine, luminescence, photocatalysis, solar cells, displaypanels, single-electron transistors, etc.[44].The surface passivation of semiconductor nanoparticles with a layer of inorganic, organic or bioactive materials forms the core-shell nanoparticles [45]. For the use of semiconductor nanoparticles as luminescentmaterials, their surface properties significantly affect theoptical properties because their surface states are known for their photodegradation and luminescent quenching sites. The surface states resemble to trap the electrons and capability to induce the non-radiative recombination of these charge carriers, leading to the reduction of luminescence efficiency. Recently, the concept of quantum confinement by the band-gap engineering of the heterostructured semiconductor nanoparticles has been suggested to increase the quantum yield or the photoluminescence intensity [44]. From this viewpoint, many semiconductor nanoparticles capped with the higher band-gap or dielectric insulation materials have been designed and synthesized in recent years such as CdSeon ZnSe, CdS on CdSe, SiO_2 on CdSe, and so on. Additionally, the coating of organic dye molecules on the semiconductor nanoparticles has potential use in solar cells[46].Therefore, the core-shellnanoparticles based on the semiconductor nanoparticles have scientific meanings including enhanced PLimproved stability against photochemical oxidation, enhanced processability and engineered band structures in the developing nanotechnology [47]. Among the semiconductor nanoparticles, ZnO nanoparticles attract our attention due to their potential applications in photocatalyst and UV laser [48, 49].

ZnO is a candidate for UV lasing materials, but its UV emission is not enough for practicability [50, 51]. Therefore, several researchers embedded ZnO nanoparticles in the higher band-gap or dielectric insulation matrixes to improve the emission intensity, such as SiO2, MgO, CaF2, BN, and polymer. Additionally, as a photocatalyst, ZnO nanoparticles could not be utilized in the acid solutions [52]. Thus, their reputation forphotocatalysis is not as well as for titania. According to the above, the surface modification of ZnO nanoparticles is necessary for their practical applications.

2.4. Synthesis of nanoparticles

Synthesis of dimensionally controlled particles inlarge quantities and study their properties toinvestigate novel applications is a preferred activityfor researchers in nanomaterials. There are several physical, chemical procedures for synthesis of ZnO nanoparticles in large quantities in a short period of time. Among them simple-solution based methods, chemical precipitation, sol-gel, solvothermal or hydrothermal, electrochemical and photochemical reduction method are preferable methods.ZnO nanoparticles can be synthesized form various species of plant leaf extract, microorganisms like bacteria and fungi cellular extract using green synthesis methods.The green synthesis of nanoparticles also governs by various enzymes. There are a lot of different methods of ZnO nanostructures preparation like metal organic vapour phase epitaxy, evaporationof high temperature, gas spraying, pulsed laser deposition, sputtering, sol-gel, wet chemical and electrochemical methods.Different applications of nanoparticles based on different protocols which have been designed for synthesis of nanoparticles [53].The different methods used for synthesis of nanoparticles are follows:

- Physical method
- Chemical method
- Biological method

2.4.1. Physical method

Physical methods developed for synthesis of ZnO nanoparticles often require special equipments or operational control. The physical method often called top-down approach which includes methods like diffusion, irradiation, thermal decomposition and arc discharge etc.The thermal decomposition method is one of the significant top-down approach. It is used for the creation of monodisperse nanoparticles. These approaches use larger initial structures or macroscopic unitswhich can be externally controlled in the processing of nanostructures. Etching through the mask; ball milling and application of severe plastic deformation are common examples of physical top-down method of synthesis of nanoparticles.The top-down method of synthesis is a model which generates on a larger scale for further reduction of nano scale. Mostly the top-down methods are not cheap and quick to manufacture. This method is slow and not suitable for large scale production.

In this method of synthesis fatty acids are allowed to dissolve in the hot NaOH solution and then mixed with the metal salt solution. After that, the solution used toformsthe metal precipitates. Crystals and short wires of copper are allowed to be encircled in the glass ampoules by the diffusion method. Then, it is sealed at low pressure followed by annealing at 5000˚C for 24 hours. Subsequently the crystals are eradicated from the ampoules. At room temperature, the ampoules free crystals arechilled on a metallic plate. In UV irradiation method, polycarbonate films are placed on glass microscope slide .Then this slide is exposed to UV radiation[53].

2.4.2. Chemical method

Moderate reaction conditions and convenient synthetic manipulation have rendered chemical methods an attractive option for accessing ZnO nanoparticles. Traditional chemical routes that are frequently used in recent years include sol-gel approach, chemical precipitation and colloidal synthesis. Surfactants or polymer based preparative method for ZnOnanomaterials are also quite popular to avoid agglomeration and achieve better control of ZnO nanoparticles. Removal of surfactants or polymers, however, poses serious limitations to such methods. Hence, delicate and precise control of experimental factors for the synthesis of nanoparticles with desired morphologies is a cherished goal for future device applications. Homogeneous chemical precipitation method is often considered economically viable for preparation of monodisperse metal oxide particles of different shapes and sizes. The method also provides better control of chemical and morphological characteristics [54].

Chemical methods, including precipitation from inorganic or organic solutions and sol-gel techniques can convenientlyprovide control of nucleation, growth and ageing of particles inthe solution. The methods rely on advanced solution andcoordination chemistry theories, enabling the synthesis of therequired precursor particles utilizing a variety of parametersto enable control of the solid formation process. A major contribution to the growth of particles is through Ostwaldripening, where small particles with lower solubility productdissolve and re-precipitate on the surface of larger particles insolution. Agglomeration takes place in solution as the particlesclog together to minimize surface energy [55].

Mostly the chemical methods of synthesis of nanoparticles are the bottom up approaches. These approaches include the miniaturization of material components (upto atomic level)with further self-assembly processes leading to the formation of nanostructures. During self-assembly the physical forces operating at nanoscale are used to combine basic units into larger stable structures. Typical examples are quantum dot formation during epitaxial growth and formation of nanoparticles from colloidal dispersion. In these methods different metal particles are reduced to form nanoparticles. Various types of metal particles used like Sodium borohydrate and Sodium citrate etc. [56]. Other chemical reagents used are N, N-dimethylformamide (DMF), Poly (N-vinyl pyrrolidine (PVP), ethyl alcohol, tetra-n-tetra-fluroborate (TFATEB), CTAB, etc. [18, 19, 20]. This method initiates with a pattern which is generate larger scale, then reduced to nano scale. This method of synthesis of nanoparticles is cheap and quick to manufacture. This method is slow and not suitable for the large scale production.

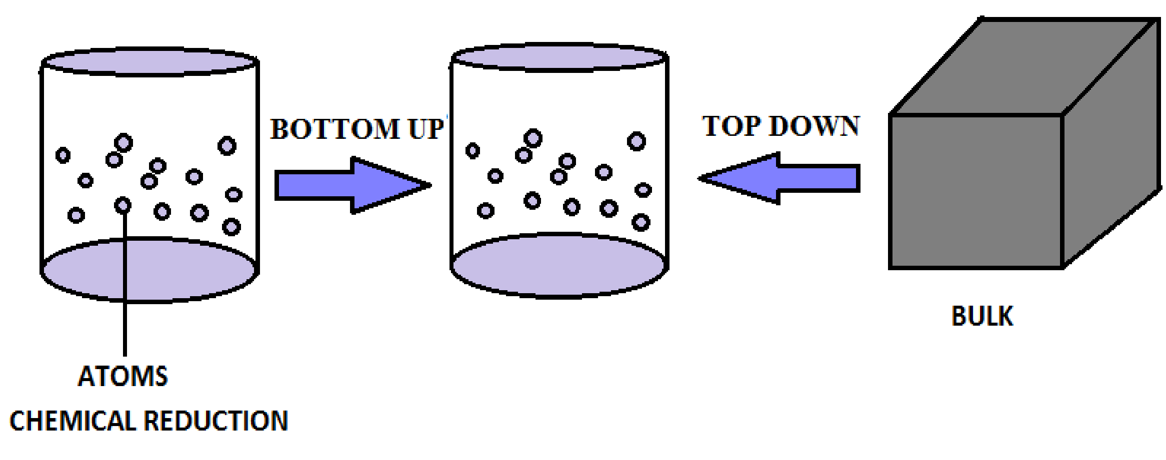

Fig. 3. Synthesis of nanoparticles by physical (top down) and chemical method (bottom up)

2.4.3. Biological method

Green synthesis method is proved as beneficial over other methods which are implemented for the synthesis of nanoparticles.Green synthesis methods are eco-friendly approach and compatible for pharmaceutical and other biomedical applications,asthe toxic chemicals are not used in these methods [57]. Besides, this method does not require high pressure, temperature. Synthesis of various nanoparticles by microorganisms is used more than the other techniques due to itsecofriendly nature. The use of environmentally beneficial materials like plant leafextract, bacteria, fungi and enzymes for thesynthesis of zinc nanoparticles proposes abundant benefits of ecofriendliness and compatibility for pharmaceutical and many other biomedical applications [2].

2.5. Application of nanotechnology

1. Solar steam light is a successful invention of nanotechnology due to the scarcity of electricity which is useful in case of the wide application of water purification and disinfecting if the dental instruments.

2. The nano engineered polymer matrix is used for light tubes with high efficiency which is shatter proof and has the capability of efficiency of compact fluorescence light bulbs.

3. The carbon nanotubes are used in the blades of windmill which are strong and also with lower weight. The blades of longer size which are filled with a huge amount of carbon nanotubes are able to produce more electricity.

4. The wires with carbon nanotubes are lower resistance than the wires of electric transmission grid. The radical change of the electricity distribution grid is now possible by nanotechnology.

5. The graphene are useful for the increase in the binding energy of hydrogen in a fuel tank with respect to the surface of the graphene layers which produces a higher amount of hydrogen for storage which is of very lighter weight for the fuel tank.

6. Sodium borohydride nanoparticles are also useful for the storage of oxygen.

7. The piezoelectric nanofibers are more flexible for woven into clothing. The fibers are capable to modify the normal motion into electricity especially for the mobile like electronic devices.

8. The energy consumption produced by friction can be reduced by the use of various lubricants which are used by the inorganic bucky balls.

9. Now-a-days nanotech solar cells are developed which are comparatively of lower cost than the conventional solar cells.

10. Currently, the batteries are also made of nanomaterials.

11. The efficiency of the membranes in fuel cells are separated by the hydrogen ions from the other gases like oxygen is now possible only for the advancement of the nanotechnology.

12. The fossil fuels like petrol, diesel and gasoline are produced by the low grade raw materials to increase the functionality of various machines and engines by the wonder of nanotechnology and it can also produce fuels from the normal raw materials which are more efficient.

13. Nanotechnology is also useful for reducing the cost of the fuel cells by changing the spacing between the platinum atoms which can be used in the fuel cells to increase the catalytic ability of the platinum. It allows the fuel cell to function with about 80% less platinum which can appreciably and considerably reduce the cost of the fuel cell.

14. The nanoclusters are valuable for the control of chemical waste materials from industries to control various pollutions. For this purpose, the nanoclusters act as catalysts to reduce the polluting waste byproducts which are generated in the process accustomed

toproduce propylene oxide. Propylene oxide is utilized for the production of common materials like plastics, paint, detergents.

15. Nanoparticles are effective in cleaning up the organic solvents which are polluting the ground water.

16. Nanostructured membranes are designed to control carbon dioxide in the exhaust stacks of power plants which cause air pollution.

17. Voltaic organic compounds are cleared from the air by using a catalyst which helps in the breakdown of voltaic organic compounds at room temperature. The catalyst consists of porous manganese oxide.

18. The nanoparticles are the miracle of nanotechnology which creates a miracle in the field of medical science especially in cancer cells.The engineered nanoparticles can be deliveringto the target site which attracts by the diseased cells for allowing the direct treatment of the cells. For example, nanoparticles which deliver chemotherapy drugs directly to the cancer cells are under development.

19.The chemotherapy drugs can also be delivered to the cancer cells with applying the heat to the cells. For example, gold nanorods to which DNA strands are attached. The DNA strands act as a scaffold which holds together the nanorods and the chemotherapy drug. When the infrared light illuminates the cancer cells, the infrared light absorbs by the gold nanorodwhich turns into heat. The produced heat releases the chemotherapy drug and destroys the cancer cells.

20. Nanoparticles are also developing to defeat the viruses. The nanoparticles delivers an enzyme that prevents the reproduction of viruses in the patient’s bloodstream instead of destroy the viruses.

21. Some nanoparticles are developed which can be delivered across the brain barrier to tackle neurologic disorders.

22. Immune response can be developed by vaccines by attaching the vaccine molecules to a DNA nanostructure which delivers the vaccine molecules to the specific cells that are in key triggering white blood cells to an immune response.

23. Sound waves are the powerful events which are mainly for the noninvasive surgery which is used by a carbon nanotubes coated lens to convert the light from a laser to focused sound waves. This developed invention by the researchers develop a method which can blast the tumor cells or to the other diseased sites without damaging the healthy tissues.

24. Bismuth nanoparticles are used for the radiation therapy for the treatment of cancer. The preliminary results specify the bismuth nanoparticles can increase the dose to the tumor by 90 percent.

25. Nanoparticles consist of polyethylene glycol-hydrophilic carbon clusters (PEG-HCC) which have been shown to absorb the free radicals at a much higher rate than the proteins out body uses for this function. They have the ability to absorb free radicals may reduce the harm which is caused by the release of the free radicals after a brain injury.

26. Targeted heat therapy is being developed to destroy the breast cancer tumors. In this methodology of treatment the antibodies are strongly attracted to proteins produced in one type of breast cancer cell which are also attracted to the nanotubes for the accumulation towards the tumor. Infrared light from a laser is absorbed by the nanotubes and also produces heat which incinerates the tumor.

27. Nanofibers can also stimulate the construction of the cartilages in the damaged joints.

28. Nanoparticles are also used in the early detection of the cancer tumors. When the nanoparticles attach to cancer tumors, the nanoparticles release the biomarkers molecules known as the peptides. In this technique, the each nanoparticle carries several peptides of a very high concentration of these markers which will occur at very early stages of cancer and also allowing the early detection of the disease.

29. The magnetic nanoparticles can attract and attach to the cancer cells in the blood stream. These types of nanoparticles may allow the doctors to easy removal of the cancer cells before they can ascertain the new tumors.

30. The early detection method for brain cancer under the development uses magnetic nanoparticles and NMR technology. The magnetic nanoparticles attach to the particles in the blood stream known as microvesicles which originate in brain cancer cells. The NMR technique is used to detect these microvesicles or magnetic nanoparticle clusters which allows the early diagnosis.

31. The iron nanoparticles can be used to improve the MRI technique of the cancer cells. The nanoparticles coated with a peptide which binds to a cancer tumor cells. Once the nanoparticles are attached to the cancer tumor cells, then the magnetic property of the iron oxide enhances the images from the MRI scan.

32.The carbon nanotubes and gold nanoparticles are being used in a sensor which detects proteins investigative of the oral cancer.

33. The nanoparticles coated with the proteins can attach to damaged portions of the arteries. This technique provides the delivery of drugs to the damaged regions of arteries to fight against the cardiovascular disease.

34. The diagnosis of early detection of the kidney damage is being developed by the use of gold nanorods which are functionalized to attach the proteins generated by the

damaged kidneys. The color of the nanorod changes when protein accumulates on the nanorod.

35. Nanorods are useful in case of the diagnostic system are being used to separate the microorganisms like viruses, bacteria and other foreign substances of blood sample allows the Raman spectroscopy signals of the components which has been demonstrated to allow the identification of viruses and bacteria in less than one hour.

36. The earliest nanomedicine application was the use of nanocrystal which acts as an antimicrobial agent for the treatment of wounds. The most common silver nanocrystal is used as an antimicrobial agent for the treatment of wounds.

37. A nanoparticle cream has been shown to fight against the infections. The nanoparticles contain nitric oxide gas is well recognized as to kill the bacteria. Research on animal model like mice have shown that the use of the nanoparticle cream releases the nitric oxide gas at the site of staph eruption significantly reduced the infections.

38. Burn dressings are treated by the nanocapsules containing antibiotics. The infection produced by the harmful bacteria in the wound causes the breaking of the nanocapsules for the release of antibiotics which allows a quicker treatment of an infection and reduces the number of frequencies of dressing.

2.6. Zinc oxide nanoparticles

Zinc oxide is an inorganic compound having formula ZnO.This is a white powder which is insoluble in water. The structural arrangement and configuration ofZnO nanoparticles has been investigated by the use of new atomistic potentials. Mechanical properties like internal stress and adhesion characteristics are required to maintain patterning accuracy and durability for various applications of nanoparticles. The ZnO is hexagonal wurtzite in structure.Generally ZnO crystallizes into hexagonal wurzite and Zinc blend. In recent years Zinc oxide has been considered as an essential semiconductor having wide band gap of 3.37 eV and large exciton-binding energy of 60 meV[58].ZnO has been marked as a highly multitasking metal oxide due to its distinctiveelectrical and optical properties [59].

Zinc oxide is often used in a number of areas of technology. It is admirable to investigate that the high quality self textured ZnO films are synthesized on different varieties of substrates [60]. The current growth in the most advanced field of porous and nanometric materials prepared by non-conventional processes which has been stimulated by the search of new and a number of applications of ZnOnanoparticulates [61]. Zinc oxide nanoparticles have been included in a number of dissimilar polymers consist of polypropylene.

2.7. Properties of nanoparticles

The semiconductors with the dimensions in the nanometer are important because of their electrical, optical and chemical properties which can be tuned by the changing the size of particles.

The properties of zinc oxide nanoparticles are classified as follows:

- Physical properties
- Mechanical properties
- Electricalproperties
- Opticalproperties
- Chemicalproperties

2.7.1. Physical Properties

ZnOhas a widebandgapof 3.3 eVsemiconductor having a greatexciton binding energy which is of 60 meV at room temperature. Thus, with its compatibility in photovoltaics and other applications, there are much information on the synthesis, growth kinetics and crystallization of ZnO through the aqueous method of direction. They are also considered as most capable applicant for nano-optoelectronics, sensors, transistors, nanopiezoelectronics and UV-detection [54]. The growth rate of a crystal is controlled by a combination of internal, structurally related factors like intermolecular bonding preferences or dislocations and external factors like supersaturation, temperature, solvents and surfactant [62]. Molecular Weight of zinc oxide is 81.37 and relative density is 5.607.Zinc oxide in pure microcrystalline form is white in color and the single crystal zinc oxide is colorless. Zinc oxide turns yellow on heating and also converts to its original color i.e. white on cooling.Melting Point of zinc oxide sublimes at atmospheric pressure at temperature over 1200˚C and under high pressure a melting point of zinc oxide is approximately 1975˚C.Vapour pressure is 12mm at temperature 1500˚C.Heat of sublimation between 1350˚C and 1500˚C is 129 Kcal/mole in which vapour is not disassociated and 193 Kcal/mole in which vapour is associated. Heat capacity (Cp) is 9.62 cal/deg/mole at temperature 25˚C and the co-efficient of thermal expansion is 4 x 10(-6)/˚C.

2.7.1.1. Crystal Structure

Zinc oxide has the wurtzite hexagonal crystal structure which is most stable. It shows the crystal structure only under the electron microscope. The distinct structure of the crystal depends on the method of formation. Zinc oxides show contrasts and discrepancy between the acicular needles and the plate shaped crystals. Zinc oxide can be induced to formulate a crystalline structure using a specific deposition method which is presentlyanespecialfield of research and development. Zinc oxide frequently crystallizes in the hexagonal wurtzite structure, zinc blende of cubic structure and also rock salt of cubicforms.

2.7.2. Mechanical Properties

Zinc oxide is a comparatively very malleable material with approximate hardness of 4.5. Its elastic constants are relatively smaller than those of other III-V semiconductors, e.g. GaN. ZnO shows many advantages such as it has a larger exciton energy approximately 60 meV than GaN which has the exciton energy approximately of 23 meV. This is useful for efficient laser UV applications. The band gap is from2.8 to 3.3 eV and from 3.3 to 4 eV in the alloys with Cd and Mg, respectively. The ZnO has the capacity of wet chemical method of synthesis. It has low power threshold at room temperature. Diluted Mn-doped ZnO shows room temperature ferromagnetism [63]. The high heat capacity and high heat conductivity, low values of thermal expansion and high melting points are some of the characteristics of ZnO. ZnO has been recommended as a more capability for UV releasing phosphor than GaN due to its higher exciton binding energy, i.e., 60 meV. Mostly all the semiconductors bonded tetrahedrally. Among all the semiconductors, ZnO has the highest piezoelectric tensor. This makes it a significant and essential material for numerous piezoelectric applications which necessitate a high degree of electro-mechanical coupling among them.

2.7.3. Electrical Properties

The fundamental study of the electrical properties of ZnO nanostructures is crucial for developing their future applications in nanoelectronics. ZnO has a moderately large band gap of 3.3 eV at room temperature. The benefits of a large band gap comprise higher values of breakdown voltages, sustaining large electric fields, high-temperature and high-power operations. ZnO has n-type character, in the absence of doping. The origin of n-type characteris typically the non-stoichiometry analysis. Due to the imperfections like the oxygen vacancies, zinc interstitials andZnO nanowires are reportedly show n-type semiconductor behavior. The major hindrance of ZnO is the broad series of applications in electronics and photonics with the complexity of p-type doping. The favorable and advantageous p-type doping for ZnO nanostructures will enormously enhance their upcoming advance applications in nanoscale electronics and optoelectronics. The p-type and n-type ZnO nanowires can serve as p-n junction diodes and the light emitting diodes (LED).

2.7.4. Optical Properties

Zinc oxide is generally transparent to visible light but strongly absorbs ultra violet light below 3655 A. The absorption is typically stronger than other white pigments. In the region of visible wavelengths, regular zinc oxide appears white, but, rutile and anatase titanium dioxide have a higher reactive index and thus has a superior opacity. ZnO is a wide band gap of 3.4 eVsemiconductor with a largeexciton binding energy of 60 meV and also considered as one of themost promising semiconductor material for electronic, photonic,optical and biological applications [64]. The band gap energy occurs between the between valence and conducting bands corresponds to the energy of 3655 photons. Zinc oxide is considered asthe photoconductive under the analytical study of ultra violet light. The arrangement of optical and semiconductor properties construct a doped zinc oxide which is a contendor for new generations of devices. Solar cells require transparent conductive coating like indium tin oxide and zinc oxide are the finest materials.

Intrinsic optical properties of ZnO nanostructures are being intensively deliberated for the implementing photonic devices. Photoluminescence (PL) spectra of ZnO nanostructures have been extensively reported. Excitonic emissions have been examined from the photoluminescence spectra of the ZnOnanorods. It is shown that quantum size confinement can significantly enhance the exciton binding energy. Strong emission peak at 380 nm due to band-to-band transition and green-yellow emission band related to oxygen vacancy are observed. Photoluminescence spectra show that the ZnO nanowire is a capableand promising material for the UV emission.Its UV lasing property is additionallymore significant and interesting. Due to its near-cylindrical geometry and large refractive index of nearly about 2.0, ZnO nanowire or nanorod is a natural candidate for the optical waveguide. The additional advantages of ZnO nanowire lasers are that the excitonic recombination lowers the threshold of lasing, and quantum confinement yields a substantial density of the band edges and enhances radiative efficiency. Optical wave guiding using dielectric nanowire also achieved considerable progress. In recent times, ZnO nanowires were stated as sub-wavelength optical waveguide. Optically forced light emission was guided by ZnO nanowire and coupled into SnO_2 nanoribbon. These discoveries show that the ZnO nanostructures can be potential building blocks for integrated optoelectronic circuits. Optical properties have a great interest towards the vast application of optoelectronics, photovoltaics and biological sensing [65].

2.7.5. Chemical Properties

Zinc oxide occurs as the mineral zincite or as white powder known as zinc white. It is usually orange or red in color due to manganese impurity.Crystalline zinc oxide is thermochromicin nature which changes from white to yellow colour when heated and turns back to white colour on cooling. This change in colour is caused by a very small loss of oxygen at high temperatures.Zinc oxide is amphoteric in nature which reacts with both the acids and alkalis. When ZnO reacts with acid, it forms zinc sulfate. Similarly whenit reacts with alkali, it forms zincates.ZnO decomposes to form zinc vapor and oxygen indicating its considerable stability. Heating of ZnOwith carbon converts the ZnO into Zn which is more volatile in nature. Exposure of zinc oxide to air absorbs both the water vapor and carbon dioxide forms zinc carbonate.

2.8. Applications of zinc oxide nanoparticles

1. It has been used as a source of zinc which is an essential trace element in food industry [66].

2. It also widely applied to various cosmetic productslike sunscreen productswhile it acts as an invisible obstaclewhich scatters UV radiation away from the skin relatively than permitting its destructive energy to be absorbed [67, 68]. But, absorption rate of zinc is low via oral ingestion and bulk-sized ZnO has poor dispersion property, resulting in low UV blocking capacity, low transparency on skin, and hard agglomeration compared to small-sized ZnO. Thus, nano-sized ZnO has recently attracted much attention in order to enhance the uptake of zinc and increase UV filtering efficiency with cosmetic clarity.

3. The similar kind of active ingredient used in the calamine lotion and diaper cream.

4. It is now generally accepted that nanoparticles efficiently penetrate the cell membrane compared to micro-sized particles. However, few researches were performed to demonstrate the effects of physicochemical parameters of ZnO nanoparticles on cellular uptake, which may provide critical information on their toxicity potential.

5.Most activities to conflictand have centered around thenanocoatings or nanocomposites using nanoparticulates of silver and zinc oxide.Their antimicrobial effects of natural biological compounds have also been demonstratedand confirmed by various researches[69].Zinc oxide show evidence of antibacterial activity which increases with decreasing the particle size [70].ZnO is nontoxic and it is a strong antibacterial agent.

6. ZnO nanoparticles have strong resistance to microorganisms and this property is due to the production of reactive oxygen species (ROS) on the surface of nanoparticles.

7.Zinc nanoparticles are used as preservative for various materials and productssuch as plastics, ceramics, glass, pigments, foods, etc. [71].

8. ZnOnanoparticles are used in industrial sectors including environmental, synthetic textiles and food packaging [28].

9. The biggest advantages of ZnO are a low price, good gas sensing properties, photocatalytic activity,antibacterial activity, possibility to prepare structures with interesting optical properties, like photonic crystals,catalytic materials, etc. [60].

10. Zinc oxide particles are used for many applications including as an activator of accelerator in the vulcanization process, as a form control in lattices, as reinforcement filler in white stocks, as an additive in lubricating oil and in catalysts.

11. Ultrafine ZnO particles show a high degree of transparency, making them useful in sunscreens, paints, varnishes, plastics and cosmetics, especially for broad UV-A and UV-B blocking [72].

12. There is evidence from the isolated by the *in vitro* cell experiments that ZnO and TiO_2 may inducefree radical formation in the presence of light and this free-radical generation may cause celldamage which is of photo-genotoxicity with ZnO.

13. Zinc oxide, a II–VI semiconductor, is particularly important because of their unique optical or electronic properties and promising applications in various fields such as photonic catalysis, light emitting diodes, field emission, gas sensors and solar cells [73].

2.9. Uncoated ZincOxide

Mostly many sunscreens contain zincoxide particles which have been coated with an inert substance. The coating of zinc oxide particles is often to make the UV ray less reactive to the skin and also make easier to mix with the other ingredients. Now-a-days, all sunscreen are the constituent of zinc oxide which is uncoated pharmaceutical grade zinc oxide. Calamine lotion and diaper rash cream also constitutes the uncoated zinc oxide. The uncoated zinc oxide is more photo reactive than the coated zinc oxide. Zinc oxide is one of only 17 active ingredients which is recently approved by the FDA for the only use of sunscreen. The zinc oxide particles deposit on the outermost layer of covering of our skin i.e. stratum corneum where they scatter to absorb ultraviolet radiation and protects our skin. Zinc oxide is a unique ingredient for sunscreen among all other sunscreen ingredients. It acts as a broad spectrum blocker which acts as asafeguard of UVA (320-400 nm), UVB (290-320 nm) and UVC [72]. Titanium dioxide (TiO_2) is a mineral active ingredient which is also used in sunscreen. It protects our skin from UVB rays in preference to UVA rays.

Skin exposure to nanoparticles containing sunscreen leads to the incorporation of TiO_2 and ZnO nanoparticles in the stratum corneum which can alter the specific nanoparticles attenuation properties due to the particle-particle, particle-skin interactions and skin-particle-light like physicochemical interactions.

2.10. Zinc oxide: Healthy or risky

Zinc oxide mostly in powder forms may cause risk for health if inhaled. It is not ananxiety with the zinc oxide in various cream and lotion based sunscreens. Zinc oxide can acts as a photoreactive substance which led to the UV exposure can generate reactive oxygen species or free radicals which can damage the living cells. It has not yet been establish with the zinc oxide in our sunscreens because:

- The rate of reactivity is still very low in comparison with the Titanium dioxide, zinc oxide nanoparticles and other active chemical sunscreens [74].
- Zinc oxide set down on the outermost layer of covering of our skin, i.e. dermis and the other cells present in the epidermis is not affected by the free radicals which are generated in the dermis.
- The inactive ingredients like organic olive oil, sunflower oil, vitamin E and more active antioxidants can scavenge and absorb the free radicals.

2.11. Minimum inhibitory concentration

The Minimum inhibitory concentration (MIC) is defined as the lowest concentration of the anti microbialwhich inhibits the visible growth of a microorganism after the overnight incubation and Minimum bactericidal concentration (MBC) as the lowest concentration of anti microbialagent which prevents the growth of a specific microorganism after the subculture on to the antibiotic free media. MIC is used by the diagnosis laboratories mainly to confirm the resistance. But most are of research tools for the determination of the *in vitro* activity of new antimicrobials and the data from the study of it used to determine the MIC break points [75].Theminimum inhibitory concentration test determines the antimicrobial property of a material against specific bacteria like microorganisms as well as for the other microorganisms also.

2.12. Antimicrobial activity

Among metal oxide powders, ZnO demonstrates very significantgrowth inhibition of a broad spectrum of bacteria. The suggestedmechanism for the antibacterial activity of ZnO is basedmainly on the catalysis of formation of the reactive oxygen species (ROS)from water and oxygen that disrupt the integrityof the bacterial membrane, although additional mechanisms havealso been suggested. Since the catalysis of radical formationoccurs on the particle surface, particles with largersurface area demonstrate stronger antibacterial activity. Consequentlyas a result, the size of the ZnO particles decreases with increasing antibacterial activity.

2.13. Possible types of characterization methods

1. Opticalprobecharacterizationtechniques
2. Electronprobecharacterizationtechniques
3. Scanningprobecharacterizationtechniques
4. Spectroscopiccharacterizationmethods
5. Ion–particlecharacterizationtechniques
6. Bulkengineeringcharacterizationmethods

CHAPTER 3

MATERIALS AND METHODS

3. MATERIALS AND METHODS

3.1. Bacterial strains

Bacillus thuringiensis, *Bacillus species*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumoniae*.

3.2. Glassware and Apparatus

All glass wares such as measuring cylinders, beakers, conical flasks, funnel, test tubes, 96 well plate, petriplates, filter paper (Whatman 40), eppendorf tubes, and microfuge tips etc. were purchased from Borosil, India.

3.3. Chemicals

For all experimental studies pure and analytical grade chemicals were used. For synthesis ofZnO nanoparticles Zinc acetatedehydratewas purchased from MERCK.Nutrient Agar and Nutrient Broth media were purchased from HIMEDIA. 1.85 gram of Nutrient Agar was dissolved in 50 ml. of double distilled water and sterilized properly at 15 Ibs in autoclave instrument. Then after cooling, it was poured into two petriplates respectively. *Bacillus thuringiensis* and *Bacillus species* strains were streaked under the aseptic condition of laminar air flow on the Nutrient Agar plates by phase streaking method for getting the single colonies. 1.3 gram of Nutrient Broth media was dissolved in 100 ml. double distilled water and sterilized properly at 15 Ibs in autoclave instrument. Then after cooling, *Bacillus thuringiensis* and *Bacillus species* strains were inoculated into separately into 50 ml. of Nutrient Broth media under the aseptic condition of laminar air flow. All those were kept for 16 hours incubation for proper growth of the bacteria. 2ml. of glycerol stock was prepared of the *Bacillus thuringiensis*strain with the proportion of 400µl of glycerol and 600µl of Nutrient Broth with bacterial culture sample. It was vortexed and kept in -80˚C for further use.

3.3.1. Preparation of solution for Minimum Inhibitory Concentration(MIC) test

50mM $Zn(CH_3CO_2)_2$solution was prepared which has the molecular weight of 219.49 g/mol. 0.065 gram/ 65 mg. of $Zn(CH_3CO_2)_2$was dissolved in 6ml of double distilled water. Then the solution was vortexed properly for proper mixing. Then the solution was filtered with

Whatman 40 filter paper.The 96 well plate was prepared under the aseptic condition of laminar air flow. Then it was incubated for overnight grow of bacteria at 37˚C. After overnight incubation, the 96 well plate was allowed for observation. The observation shown and proved that the bacteria, *Bacillus thuringiensis* can resists at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$.

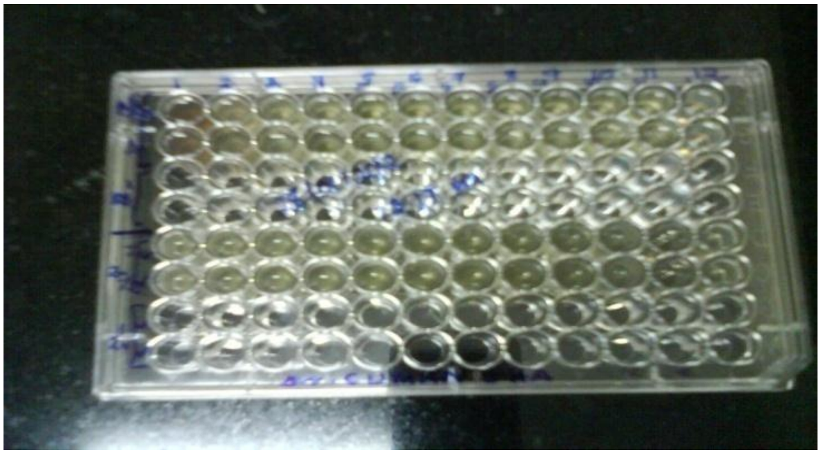

Fig.4. 96 well plate for MIC test

Table.2. Target MIC

	1	2	3	4	5	6	7	8	9	10	11	12
Resultant concentration of $Zn(CH_3CO_2)_2$ (in mM)	5	2.5	1.25	0.625	0.3125	0.1562	0.0781	0.039	0.019	0.009	Positive control	Negative control
Nutrient Broth media (in µl)	150	150	150	150	150	150	150	150	150	150	150	150
Bacillus thuringiensis culture (in µl)	20	20	20	20	20	20	20	20	20	20	20	0
50mM $Zn(CH_3CO_2)_2$	30	15	7.5	3.75	1.875	0.9375	1.872	0.936	1.824	0.912	0	30
Nutrient Broth media (in µl)	100	115	122.5	126.25	128.125	129.07	128.12	129.07	128.128	129.07	130	120

3.4. Synthesis of ZnOnanoprticles

3.4.1. Preparation of $Zn(CH_3CO_2)_2$ solution for synthesis of Zinc nanoparticles

1.3 gram of Nutrient Broth media was dissolved in 100 ml. double distilled water and sterilized properly at 15 Ibsby the help of autoclave. *Bacillus thuringiensis* strains were inoculated into the Nutrient Broth media (cooled) under the aseptic condition of laminar air flow. 0.1562mM concentration of $Zn(CH_3CO_2)_2$ of 1.71mg was added to the Nutrient Broth media. Then it was incubated at 37˚C for overnight for the growth of bacteria. After overnight incubation, the appearance of tubidity confirms the successful growth of *Bacillus thuringiensis*. *Bacillus thuringiensis* culture sample was centrifuged at 5000 rpm for 15 minutes. After centrifugation, supernatant and pellet was separated. Then the separated supernatant and pellet sample was allowed for SEM, UV, XRD and FTIR measurements. Then the separated pellet solution was mixed with deionized water and was vortexed properly for preparation of the pellet solution. Then both the supernatant and pellet samples were stored in -80˚C for further use for characterization of zinc nanoparticles.

3.4.2. Preparation of solid powder sample

The supernatant was kept in the petriplates and it was allowed to dry for the preparation of solid powder sample in the hot air oven at 80˚C for 24 hours. After that the solid powder sample was prepared and was appropriate for preparation of slides for characterization.

3.4.3. Preparation of slide for SEM analysis by fixing method

3.4.3.1. Preparation of SEM slide of the supernatant sample

After synthesis of ZnO nanoparticles, the separated supernatant was taken for SEM analysis. One drop of supernatant sample was taken by the micropipette and placed on the SEM slide. For the preparation of the slide, an another slide was placed with the angle of 45˚ on the main slide which was with the sample and by the help of placed slide was smear was produced by dragging it once. Then the supernatant sample slide for SEM analysis was allowed to dry in the room temperature (25˚C). Then the slide was preferable for the SEM analysis.

3.4.3.2. Preparation of SEM slide of the pellet sample

The pellet sample was kept in a falcon tube and was allowed for centrifugation for 15 minutes at 5000 rpm. After centrifugation, the sample was washed with PBS wash of strength 1X. Then after washing, 25% glutaraldehyde was added to it. Then the sample was allowed for 24 hours incubation at 37˚C. After overnight incubation, again the sample was allowed for centrifugation at 5000 rpm for 15 minutes. Then, the supernatant was discarded and the harvested pellet was mixed with 30% alcohol which was vortexed properly for proper mixing. Then the solution was allowed for centrifugation at 5000 rpm for 15 minutes. Then, the supernatant was discarded and the harvested pellet was mixed with 50% alcohol which was vortexed properly for proper mixing. Then the solution was allowed for centrifugation at 5000 rpm for 15 minutes. The supernatant was discarded and the harvested pellet was mixed with 70% alcohol which was vortexed properly for proper mixing. Again the solution was allowed for centrifugation at 5000 rpm for 15 minutes. Then, the supernatant was discarded and the harvested pellet was mixed with 90% alcohol which was vortexed properly for proper mixing. Then lastly,the solution was allowed for centrifugation at 5000 rpm for 15 minutes. The properly washed pellet was fixed and placed on the glass slide by the help of a microfuge tips. The prepared slide was kept in the petriplates under the protective layer of tissue paper also. Then the pellet sample slide for SEM analysis was allowed to dry in the room temperature (25˚C). Then the slide was preferable for the SEM analysis.

3.5. Culture preparation for Antimicrobial Activity test

80ml Nutrient Agar media was prepared with synthesized ZnO nanoparticles at the concentration of 200 µg/ml which refers to the volume 16 mg of ZnO nanoparticles per 80 ml. 80ml of Nutrient Agar media was prepared without ZnO nanoparticles. Thses are allowed for proper sterilizationat 15 Ibsby the help of autoclave. Then, they are poured into petriplates under the aspectic condition of laminar air flow. Then after cooling, media was solidified. Then, *Staphylococcus aureus*, *Escherichia coli*, *Bacillus species*, *Klebsiella pneumoniae* strains were spreaded on the plates with four control plates which were without ZnO nanoparticles for the comparsion of growth of the bacteria with ZnO nanoparticles and without ZnO nanoparticles for its antimicrobial property test. Then the plates were incubated at 37˚C for 16 hours for the growth of the bacteria. After 16 hours, observation was taken of the plates.

3.6. Instrumentation

JSM-6480 LV SEM instrument was employed to study the morphology of synthesized nanoparticles which was performed at an accelerating voltage of 20 kV. UV-Visible absorption spectrum was measured by Perkin-Elmer UV-Vis spectrophotometer, Lamda.35 at ordinate mode of A, slit width of 1nm, and scan speed of 240nm/win. Number of cycle was set to 1 per 1 second and lamp change value was set at 326nm.Philips PAN analytical machine was employed for X-ray diffraction studies with scanning range of 20˚-80˚ and bond angle of 3˚.The synthesized ZnO nanoparticles were characterised by Perkin Elmer Spectrophotometer FT-IR Spectrum One for the study and characterization of the attached functional groups to the surface of the synthesized ZnO nanoparticles with the scanning range of 0- 4000$cm^{-1}$the resolution of4cm^{-1}.

3.7. Characterization of ZnO nanoparticles

3.7.1. Scanning electron microscopy

In this research work JSM-6480 LV SEM machine was employed to study the morphology of synthesized nanoparticles. The experiment was performed at an accelerating voltage of 20 kV. The slide was coated with platinum and after the platinum coating, the SEM image was taken.

3.7.2. UV-Vis spectroscopy

The synthesized ZnO nanoparticles were characterised by Perkin-Elmer UV-Vis spectrophotometer, Lamda.35, at ordinate mode of A, slit width of 1nm, and scan speed of 240nm/win. Number of cycle was set to 1 per 1 second and lamp change value was set at 326 nm for the determination of the Plasmon resonance property of ZnO nanoparticles, reduction of metal ion and formation of nanaoparticles.

3.7.3. X-ray diffraction

In this research work Philips PAN analytical machine was employed for X-ray diffraction studies. The phase variety and particle size was determined in X-ray diffraction studies with scanning range of 20˚-80˚ and bond angle of 3˚. The powder sample was taken for the XRd analysis which was also placed on a glass slide for analysis.

3.7.4. FTIR spectroscopy

The synthesized ZnO nanoparticles were characterised by Perkin Elmer Spectrophotometer FT-IR Spectrum One for the study and characterization of the attached functional groups to the surface of the synthesized ZnO nanoparticles with the scanning range of 4000-400cm^{-1}the resolution of4cm^{-1}. For the FTIR characterization, the sample was mixed with solid KBr uniformly and properly which was compressed to settle down on a thin transparent film and this thin transparent film was for FTIR analysis which was kept in the chamber of the instrument for scanning.

CHAPTER 4

RESULTS AND DISCUSSION

4. RESULTS AND DISCUSSION

4.1. Minimum Inhibitory Concentration(MIC)

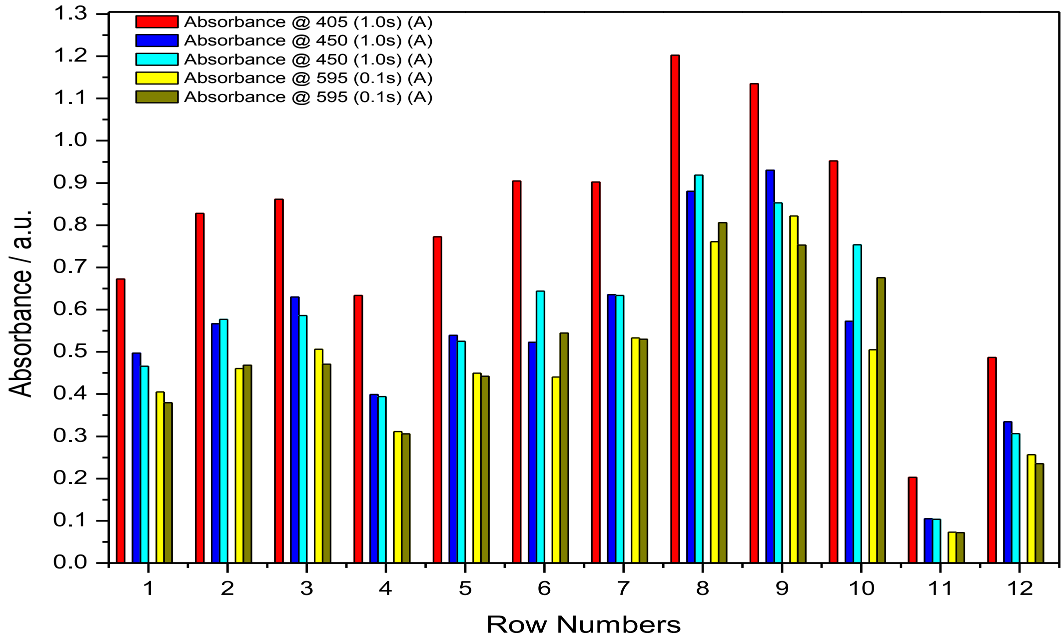

Fig. 5.Minimum Inhibitory Concentration (MIC) Test

The result of minimum inhibitory concentration test showed the resistance of bacteria at a particular concentration of 0.1562mM of $Zn(CH_3CO_2)_2$. After the MIC test, the entire protocol of the research project work of synthesis of ZnO nanoparticles was based on the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$.

4.2. Synthesis and characterization of ZnO nanoparticles

By the green synthesis method, ZnO nanoparticles synthesized successfully.During exposure to bacterial cell extracts, reduction of zinc ions into zinc nanoparticles was monitored as a result of the colour changewhichoccure due to the Surface Plasmon Resonance phenomenon. Generally the metal nanoparticles have free electrons, which helps in the formation of the Surface Plasmon Resonance absorption band.It happens due to the united vibration of the electrons of metal nanoparticles in resonance with light wave.

4.3. Characterization of ZnO nanoparticles

The synthesized nanoparticles characterized through the instrumental analysis such as Scanning Electron Microscope (SEM), UV-Visible spectroscopy, X-Ray Diffraction (XRD) and FTIR (Fourier Transform Infra-red Spectroscopy) which are follows with their scanning images.

4.3.1. SEM analysis of ZnO nanoparticles of MIC test

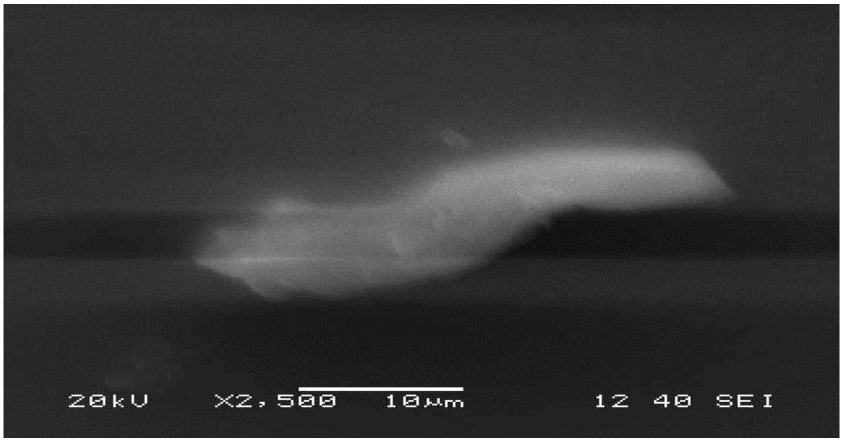

Fig. 6. SEM analysis of MIC test of supernatant sample for the determination of formation of bacterial biomass at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$

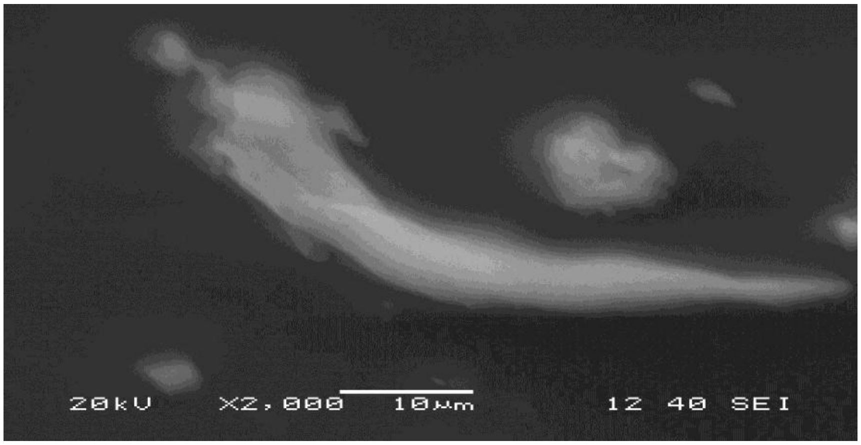

Fig. 7. SEM analysis of MIC test of supernatant sample for the determination of formation of bacterial biomass at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$

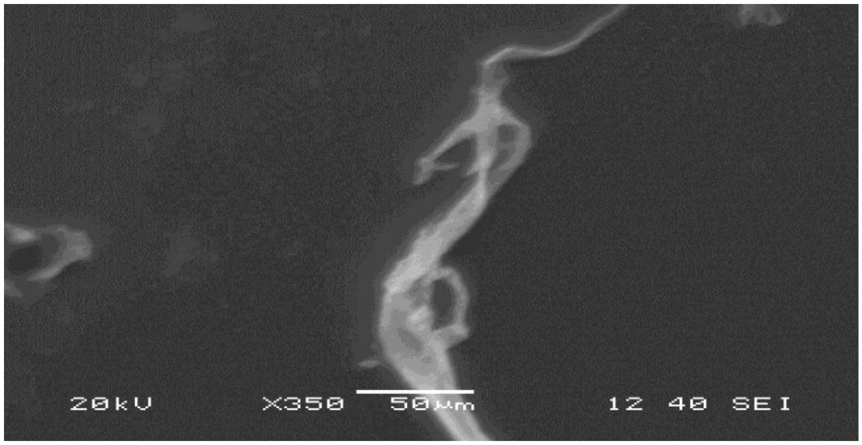

Fig. 8. SEM analysis of MIC test of pellet sample for the determination of formation of bacterial biomass at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$

The SEM analysis of pellet samples of ZnO nanoparticles shows the presence of bacterial biomass in pellet at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$. Similarly, the supernatant sample also shows the presence of bacterial biomass at the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$along with the embedded ZnO nanoparticles.SEM analysis of the MIC test by scanning both pellet and supernatant sample shows the presence of nanoparticles embedded in the bacterial biomass. The entire protocol of the research project work of synthesis of ZnO nanoparticles was based on the concentration of 0.1562mM of $Zn(CH_3CO_2)_2$ because in that concentration bacteria survived and produced the nanoparticle.

4.3.2. SEM analysis of ZnO nanoparticles

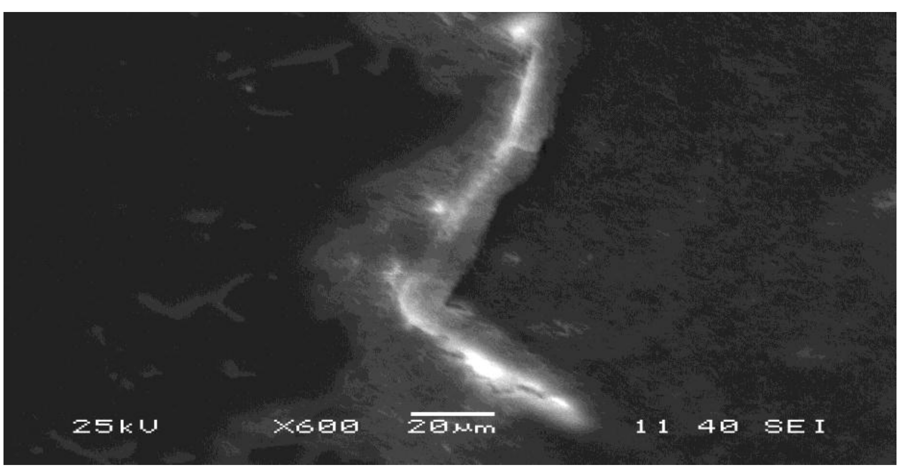

Fig. 9. SEM analysis of pellet sample of ZnO nanoparticles

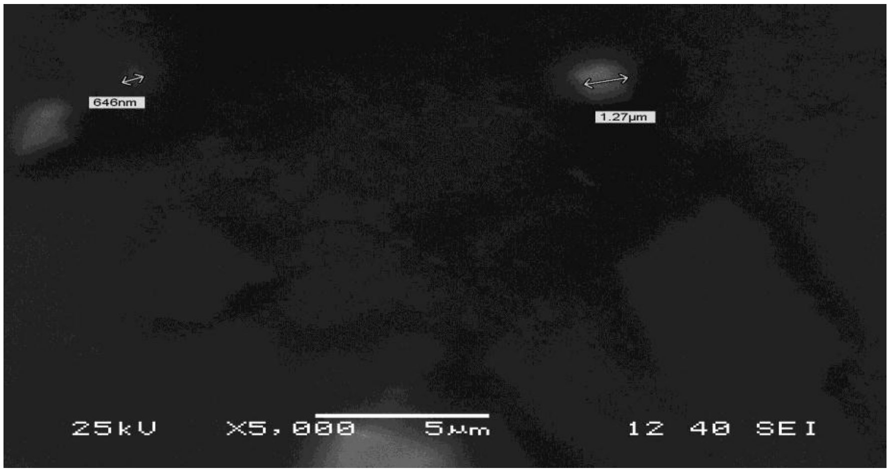

Fig. 10. SEM analysis of supernatant sample of ZnO nanoparticles

After the synthesis, the sample was centrifuged to separate the supernatant and the pellet and both they were under goes the SEM analysis by preparation of the SEM slides by fixing method of these samples.

The Figure 9 and 10 ofSEM analysis of pellet sample of ZnO nanoparticles shows the particle size of 20µm. Similarly, the supernatant sample shows the particle size 646 nm and 1.27 µm.The particles are spherical in shape. Thus, the green method is producing polydispersed nanoparticle of varying sizes.

4.3.3. UV absorption analysis of ZnO nanoparticles

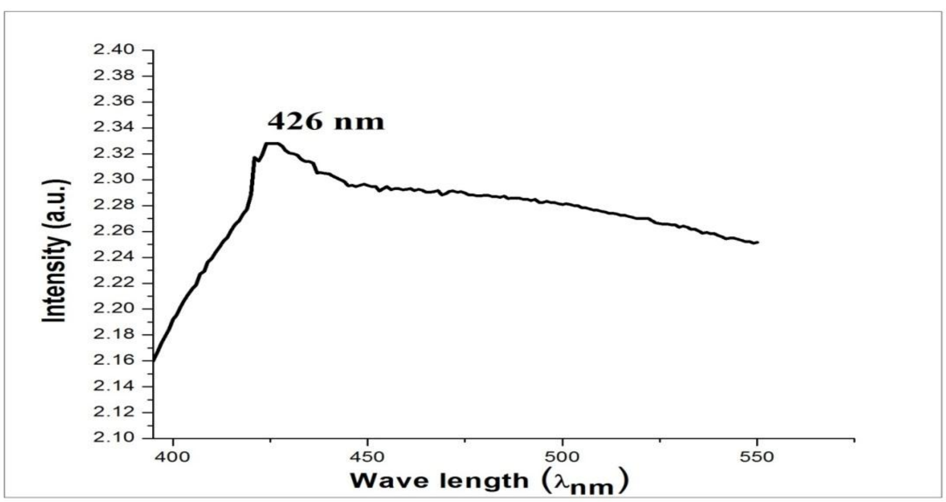

Fig.11.UV analysis spectrum of pellet sample of ZnOnanoparticles

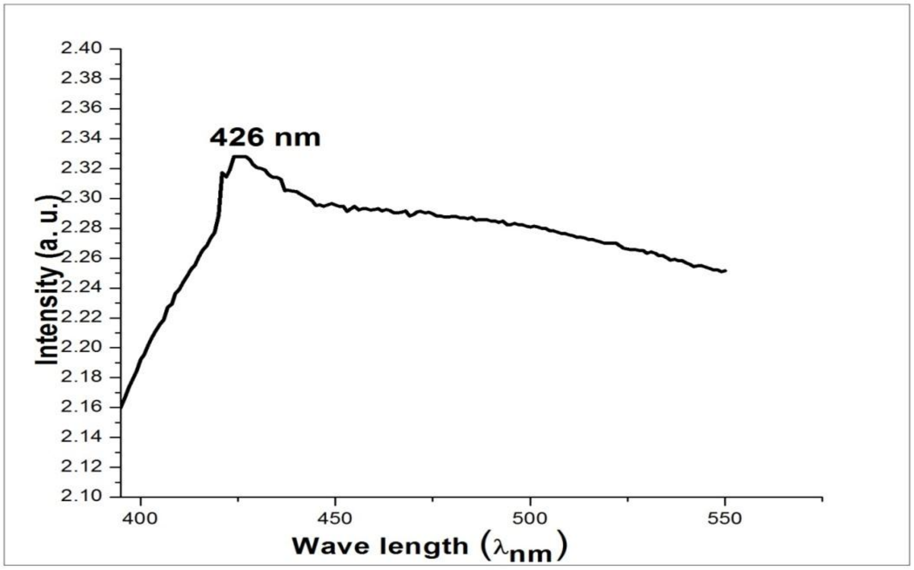

Fig.12.UV analysis spectrum of supernatant sample of ZnOnanoparticles

Figure 11 and 12 shows the UV-Vis absorption spectra of ZnO nanoparticles.The UV-Vis spectroscopic study shows the Plasmon resonance property, confirmed the reduction of metal ion and formation of nanoparticle with peak at 426 nm.The ZnOnanoparticles comprises distinguish colour in colloidal solution due to its miniature dimension. The sharp bands of zinc colloids were observed at 426 nm, whichproves that the zinc ion isefficiently reduced by the bacterial cell extract. On the surface of nanoparticles,the electron clouds are present which are able to oscillates and absorbs the electromagnetic radiation at a particular energy, energy corresponding to the photons of 426 nm. The resonance known as surface plasmon resonance.

4.3.4. XRD analysis of ZnO nanoparticles

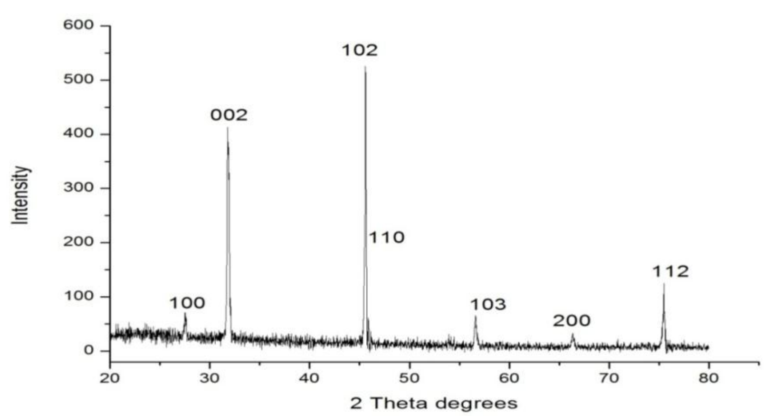

Fig. 13. XRD analysis powder sample of supernatant of ZnO nanoparticles

Figure 13 shows the distinct diffraction peaks arround46˚. The XRD study confirms the wurtzite structure of ZnO nanoparticles and the formation of narrow peak with the Bragg's angle of 2θ=102˚ suggests the crystalline nature of the ZnOnanoparticle.At the Bragg's angle of 2θ=100˚ suggests the Face centered cubic nature of the ZnO nanoparticle. Stabilization of the nanoparticles occur by some capping agents which are confirmed by the sharp peaks. The intense Bragg's angle reflection suggests the strong X-ray scattering centres in the crystalline phase which is due to the capping agents.

4.3.5. FTIR analysis of ZnO nanoparticles

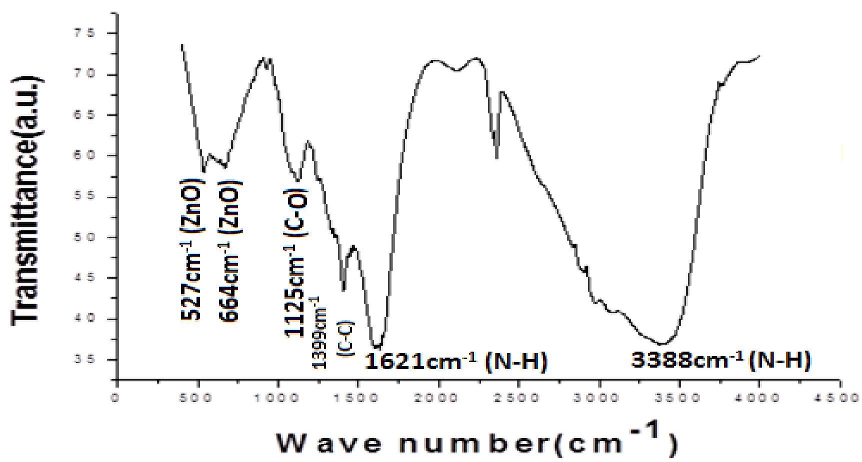

Fig. 14.FTIR analysis of powder sample of ZnO nanoparticles preparaed from supernatant

Figure 14 of FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by the microbes. It also confirms the reduction of metal ions by the used bacteria by the observed peaks are 527 cm^{-1}, 664 cm^{-1}, 1125 cm^{-1}, 1399 cm^{-1}, 1621cm^{-1}, and 3388cm^{-1}.The stretch for ZnO nanoparticles were found to be around 527 cm^{-1} and 664 cm^{-1}. The peak around 1320–1000 cm^{-1} leads toC-O stretching alcohols, carboxylic acids, esters, ethers.Here the C-O stretching alcohols, carboxylic acids, esters, ethers show the peak at 1125 cm^{-1}.1500-1400 cm^{-1}leads to C-C stretch (in-ring) aromatics. Here the C-C stretch (in-ring) aromatics show the peak at 1399 cm^{-1}. The peak around 1650–1580 cm^{-1} leads to N–H bend primary amines. Here the observed peak is 1621 cm^{-1} leads to N–H bend primary amines. The peak around 3400–3250 cm^{-1}leads to N–H stretching for primary, secondary amines, amides.Here theobserved peak is 3388cm^{-1}leads toN–

H stretching for primary, secondary amines, amides.Thus, the synthesized ZnO nanoparticles are surrounded by proteins and metabolites like terpenoids with the attachment of the various functional groups. The FTIR analysis confirmed the presence of carbonyl groups from the amino acid residues and proteins has the stronger capability to bind the metal designating the proteins which canprobably from the metal nanoparticles, i.e. capping of zinc oxide nanoparticles which suggests the biological molecules has the capability to perform dual functions of formation and stabilization of ZnO nanoparticles.

4.3.6. Antimicrobial activity test

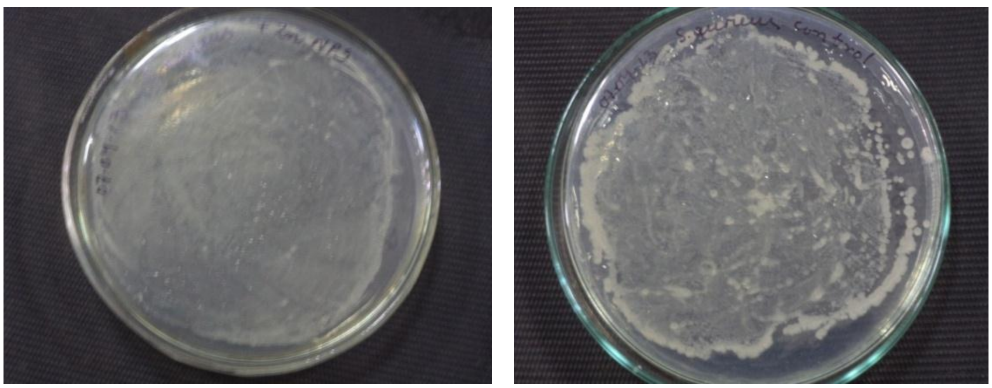

Fig. 15. A. *Staphylococcus aureus* strain with Zinc nanoparticles, B. Control plate of *Staphylococcus aureus* strain without Zinc nanoparticles

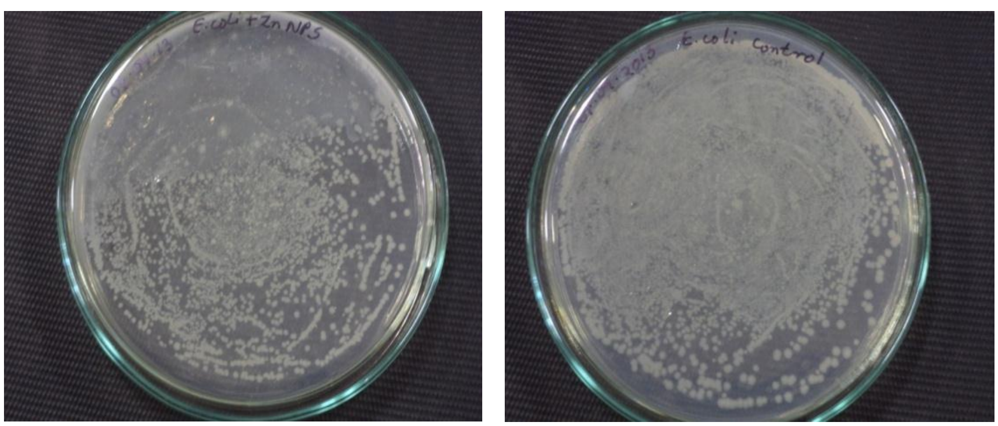

Fig. 16. A. *Escherichia coil* strain with Zinc nanoparticles, B. Control plate of *Escherichia coli* strain without Zinc nanoparticles

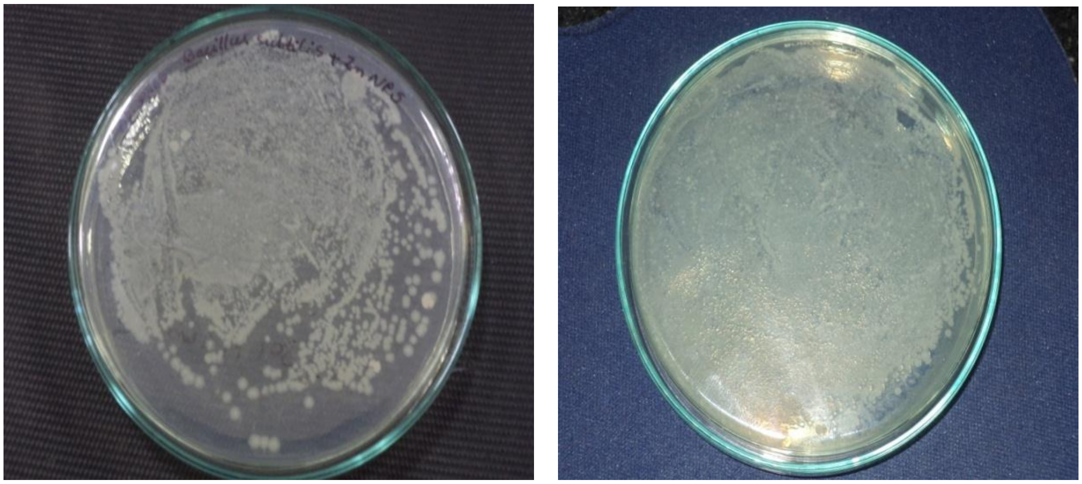

Fig. 17. A. *Bacillus subtilis* strain with Zinc nanoparticles, B. Control plate of *Bacillus subtilis* strain without Zinc nanoparticles

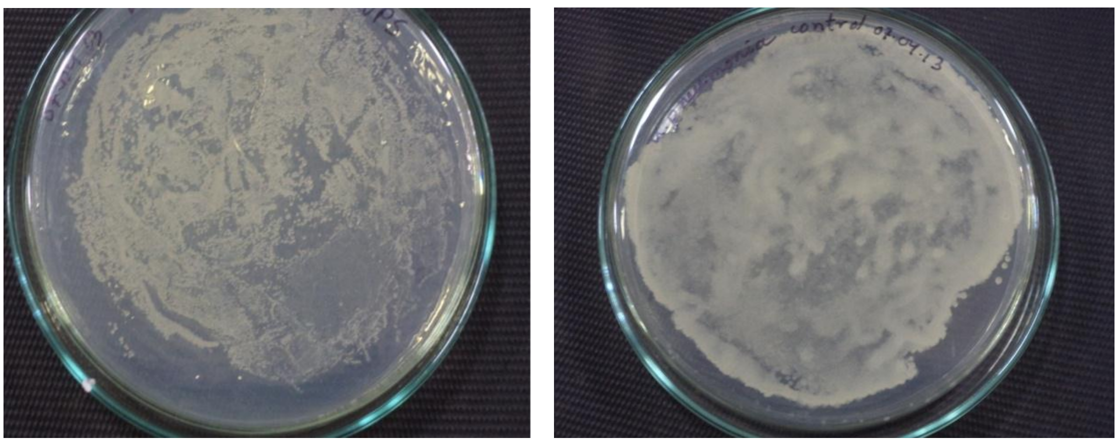

Fig. 18. A. *Klebsiella pneumoniae*strain with Zinc nanoparticles, B. Control plate of *Klebsiella pneumoniae*strain without Zinc nanoparticles

In figure 15, figure ‘A’ shows *Staphylococcus aureus* strain agar plate with ZnO nanaoparticles, and figure ‘B’ shows *Staphylococcus aureus* strain agar plate without ZnO nanaoparticles which proves that in the presence of ZnO nanoparticles the growth of bacteria is significantly less with contrast to the control plate that is without ZnO nanoparticles.In figure 16, figure ‘A’ shows *Escherichia coli* strain agar plate with ZnO nanaoparticles and figure ‘B’ shows *Escherichia coli* strain agar plate without ZnO nanaoparticles which proves that in the presence of ZnO nanoparticles the growth of bacteria is significantly less with contrast to the control plate which is without ZnO nanoparticles. In figure 17, figure ‘A’ shows *Bacillus species* strain agar plate with

ZnO nanaoparticles and figure ‘B’ shows *Bacillus species* strain agar plate without ZnO nanaoparticles which proves that in the presence of ZnO nanoparticles the growth of bacteria is significantly less with contrast to the control plate which is without ZnO nanoparticles.In figure 18, figure ‘A’ shows *Klebsiella pneumoniae* strain in the agar plate with ZnO nanaoparticles and figure ‘B’ shows *Klebsiella pneumonia* strainagar plate without ZnO nanaoparticles which proves that in the presence of ZnO nanoparticles the growth of bacteria is significantly less with contrast to the control plate which is without ZnO nanoparticles. From this above observed antimicrobial activity test, it is proved that the ZnO nanoparticle is a good and strong antimicrobial agent.

ZnO nanoparticles also have bactericidal effects on both gram-positive and gram-negative bacteria. ZnO nanoparticles have bactericidal effects on both gram-positive and gram-negative bacteria. They also have antibacterial activity against spores that are resistant to high temperature and high pressure. The antimicrobial agents are natural or synthetic compounds which are helpful in the inhibition of microbial growth.

CHAPTER 5

CONCLUSION

5.CONCLUSION

Zinc oxide nanoparticles were successfully synthesized by using biological green synthesis method using *Bacillus thuringiensis* bacteria cell extract. The SEM analysis confirms the formation of ZnO nanoparticles with spherical size of diameter ranging 646 nm to 1.27µm. The UV-Vis spectroscopic study shows the Plasmon resonance property, confirmed the reduction of metal ion and formation of nanoparticle with plasma resonance peak at 426. The ZnO nanoparticles comprises distinguishing colour in colloidal solution due to its miniature dimension. The sharp bands of zinc colloids were observed at 426 nm proves the zinc metal reduces the bacterial cell extract more efficiently. The XRD study confirms the structure of ZnO nanoparticles and the formation of narrow peak with the Bragg's angle of $2\theta=102^{\circ}$ suggests the crystalline nature and wurtzite structure of ZnO nanoparticles. At the Bragg's angle of $2\theta=100^{\circ}$ suggests the face centered cubic nature of the ZnO nanoparticles. Stabilization of the nanoparticles occur by some capping agents which are confirmed by the sharp peaks. The intense Bragg's angle reflection suggests the strong X-ray scattering centres in the crystalline phase which is due to the capping agents. FTIR measurements were carried out to identify the possible biomolecules responsible for capping and efficient stabilization of the metal nanoparticles synthesized by the microbes. It also confirms the reduction of metal ions by the used bacteria by the observed peaks are 527 cm^{-1}, 664 cm^{-1}, 1125 cm^{-1}, 1399 cm^{-1}, 1621cm^{-1}, 3388cm^{-1} . The stretch for ZnO nanoparticles were found to be around 527 cm^{-1} and 664 cm^{-1}. Here the observed peak is C-O stretching alcohols, carboxylic acids, esters, ethers show the peak at 1125 cm^{-}1; C-C stretch (in-ring) aromatics show the peak at 1399 cm^{-1},1621 cm^{-1} leads to N-H bend primary amines and3388 cm^{-1} leads to N–H stretching for primary, secondary amines, amides. Thus, the synthesized ZnO nanoparticles are attached or capped by proteins and metabolites.

The FTIR analysis confirmed the presence of carbonyl groups from the amino acid residues and proteins has the stronger capability to bind the metal designating the proteins which can probably from the metal nanoparticles

CHAPTER 6

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6. REFERENCES

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